

1

COMPOSITIONS AND METHODS FOR SILENCING SMAD4

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. provisional application 61/579,601 filed Dec. 22, 2011, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is TEKM_075_01US_ST25.txt. The text file is 6 KB, was created on Dec. 21, 2012, and is being submitted electronically via EFS-Web.

BACKGROUND OF THE INVENTION

I. Field of the Invention

The present invention relates to compositions comprising therapeutic nucleic acids that target SMAD4 gene expression, lipid particles comprising one or more of the therapeutic nucleic acids, methods of making the lipid particles, and methods of delivering and/or administering the lipid particles (e.g., for treating anemia of inflammation in humans).

II. Description of the Related Art

Anemia of inflammation (sometimes called anemia of chronic disease, or anemia of inflammatory response) is a form of anemia seen in chronic illness (e.g. chronic infection, chronic immune activation, or malignancy), and is the most common anemia found in hospitalized patients. Anemia of inflammation is abbreviated herein as "AI". Although there may be more than one underlying cause of AI, it appears likely that the syndrome is largely the result of the production of hepcidin, a protein that regulates human iron metabolism. It is believed that in response to inflammatory cytokines, such as IL-6, the liver produces increased amounts of hepcidin which, in turn, prevents the protein ferroportin from stimulating release of stored iron.

Ideally, AI is resolved by successful treatment of the chronic disease with which the AI is associated. Unfortunately, such chronic conditions may be refractory to treatment, and many patients live with AI as part of their overall health problems. In more severe cases, blood transfusions, or commercially-produced erythropoietin, can be helpful in some circumstances, although both treatments are costly, and may be dangerous (see, e.g., Zarychanski R, Houston D. S., *Can. Med. Assoc. J.* 179 (4): 333-7 (2008)). Intravenous infusion of iron has also been used to treat AI, although the iron compounds that are infused may be potent oxidants which are potentially harmful to the body (see, e.g., R. A. Zager, *Clin. J. Am. Soc. Nephrol.* 1 Suppl 1: S24-31 (Sep. 2006)).

Thus, there is a continuing need for compositions and methods for treating, preventing, and/or ameliorating one or more symptoms of AI.

BRIEF SUMMARY OF THE INVENTION

SMAD4 is a 552-amino acid protein involved in cell signaling. SMAD4 binds receptor-regulated SMADs (R-SMADs), such as SMAD1 and SMAD2, and facilitates the translocation of the heteromeric complex into the nucleus where the complex binds to DNA and serves as a transcription

2

factor. For example, SMAD4 modulates the activity of members of the TGF β protein superfamily, consequently SMAD4 is involved in many cell functions, such as differentiation, apoptosis, gastrulation, embryonic development and the cell cycle.

Wang et al. proposed a role for the SMAD4 protein in the regulation of iron metabolism. (Wang et al., *Cell Metabolism*, Vol. 2, pp. 399-409 (Dec. 2005)). They showed that liver-specific disruption of the SMAD4 gene results in markedly decreased hepcidin expression and a consequent accumulation of iron in many organs. They postulated a role for SMAD4, acting through modulation of TGF β , in regulating hepcidin expression and thus iron homeostasis.

Accordingly, it is an object of the present invention to provide compositions and methods for inhibiting the expression of the SMAD4 gene. Inhibition is through the mechanism of RNA interference. The compositions and methods of the present invention are thus useful, for example, for treating anemia of inflammation in a human being.

Thus, the present invention provides compositions comprising therapeutic nucleic acids such as interfering RNA (e.g., dsRNA such as siRNA) that target SMAD4 gene expression, lipid particles comprising one or more (e.g., a cocktail) of the therapeutic nucleic acids, methods of making the lipid particles, and methods of delivering and/or administering the lipid particles (e.g., for treating anemia of inflammation).

More particularly, the invention provides compositions comprising unmodified and chemically modified interfering RNA (e.g., siRNA) molecules which inhibit or silence SMAD4 gene expression. The present invention also provides serum-stable nucleic acid-lipid particles (e.g., SNALP) and formulations thereof comprising one or more (e.g., a cocktail) of the interfering RNA (e.g., siRNA) described herein, a cationic lipid, and a non-cationic lipid, which can further comprise a conjugated lipid that inhibits aggregation of particles. Examples of interfering RNA molecules include, but are not limited to, double-stranded RNA (dsRNA) such as siRNA, Dicer-substrate dsRNA, shRNA, aiRNA, pre-miRNA, and combinations thereof.

In one aspect, the present invention provides an interfering RNA that targets SMAD4 gene expression, wherein the interfering RNA comprises a sense strand and a complementary antisense strand, and wherein the interfering RNA comprises a double-stranded region of about 15 to about 60 nucleotides in length. In certain embodiments, the present invention provides compositions comprising a combination (e.g., a cocktail) of at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, or more interfering RNA molecules that target the same and/or different regions of the SMAD4 genome. The interfering RNA of the invention are capable of inhibiting or silencing SMAD4 gene expression in vitro and in vivo.

A non-limiting example of a SMAD4 transcript sequence that can be used, for example, in the design of siRNA molecules that inhibit SMAD4 gene expression is set forth in Genbank (www.ncbi.nlm.nih.gov/genbank) as Accession No. NM_005359.5 (Gene ID: 4089. Official Symbol: SMAD4, and Name: SMAD family member 4 [Homo sapiens]).

In another aspect, the present invention provides an interfering RNA that targets SMAD4 gene expression, wherein the interfering RNA comprises a sense strand and a complementary antisense strand, and wherein the interfering RNA comprises a double-stranded region of about 15 to about 60 nucleotides in length. In certain embodiments, the present invention provides compositions comprising a combination (e.g., a cocktail) of at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, or